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Intranucleus Accumbens Amphetamine Infusions Enhance Responding Maintained by a Stimulus Complex Paired With Oral Ethanol Self-Administration

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SLAWECKI, C. J., H. H. SAMSON AND A. CHAPPELL. *Intranucleus accumbens amphetamine infusions enhance responding maintained by a stimulus complex paired with oral ethanol self-administration.* PHARMACOL BIOCHEM BEHAV **58**(4)1065–1073, 1997.—Six male Long–Evans rats were trained to self-administer 10% ethanol (v/v) during 30 min operant sessions. A licking response on an empty drinking tube resulted in the presentation of reinforcement from an automatic dipper. During the initiation of ethanol self-administration, a tone–light stimulus complex was paired with all ethanol presentations. When 10% ethanol maintained responding, guide cannulae aimed at the nucleus accumbens (NAcc) were implanted into the brain. The ability of the paired stimulus complex to reinforce a new operant response (i.e., a lever press) was then examined. To test for the development of the new response, responding on one lever resulted in presentation of only the paired tone–light stimulus complex (contingency-associated lever) while responding on an alternate lever had no programmed consequences (no contingency-associated lever). Prior to some new response sessions, amphetamine (5–20 μg/μl) was infused into the NAcc to examine the influence of dopamine on responding maintained by the stimulus complex. Ethanol intake during the sessions prior to new response testing averaged 0.49 ± 0.07 g/g. During new response sessions no significant differences in lever pressure during no-drug conditions (control, sham, injection or vehicle injection) were observed between the contingency-associated and no contingency-associated levers. Intra-NAcc infusion of amphetamine (5–20 μg/μl) resulted in significant increases in lever pressing only on the contingency-associated lever. These data suggest that increasing NAcc dopamine levels with amphetamine enhanced the ability of the stimulus complex to function as a reinforcer. Further studies examining the ability of potentially more salient stimuli (i.e., taste of ethanol) to function as conditioned reinforcers associated with ethanol self-administration are warranted due to the apparent inability of the paired tone–light stimulus complex to function as a reinforcer without amphetamine-induced activation of the NAcc. © 1997 Elsevier Science Inc.

Ethanol self-administration Amphetamine Nucleus accumbens New response Conditioned reinforcement

CONDITIONED stimuli have been hypothesized to play a role in alcohol abuse (32,61,65). The primary focus of research examining the influence of conditioned stimuli on alcohol abuse has been on the ability of these stimuli to elicit conditioned tolerance (13) or conditioned withdrawal (32). As a result, few studies have directly assessed the ability of ethanol to establish a neutral environmental stimulus as a conditioned reinforcer (CS^R). However, Smith et al. (56) have reported that a buzzer paired with intragastric ethanol self-administration prolonged responding when presented contingent on lever pressing during extinction. This suggests that the buzzer functioned as a CS^R. In recent years, models of oral ethanol self-administration have been developed that establish voluntary consumption of behaviorally active doses of ethanol (1,20,51). These new procedures, which more adequately model human alcohol drinking, make if feasible to examine the relationship between CS^Rs and oral ethanol consumption.

Oral ethanol self-administration (25,52) and responding

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maintained by CS^Rs (30,31,59,60,66) are influenced similarly by dopaminergic activity in the nucleus accumbens (NAcc). Samson et al. (52) reported that intra-NAcc infusions of amphetamine increased responding maintained by 10% ethanol in rats. Taylor and Robbins (59,60) and others (30,31,66) have also reported that intra-NAcc amphetamine infusions increase responding maintained by CS^R over the same dose range employed in ethanol self-administration studies (25,52). Furthermore, the D_2-like dopamine receptor antagonist raclopride decreases responding maintained by 10% ethanol (25,52) and attenuates the increases in CS^R-maintained responding induced by intra-NAcc amphetamine infusions (66). Thus, like many natural reinforcers and drugs of abuse (26, 44,50), activity of the mesolimbic dopamine system influences both ethanol reinforcement and conditioned reinforcement. An understanding of how NAcc dopaminergic activity modulates reactivity to environmental stimuli during ethanol self-administration may provide insight into the interaction of CS^Rs with self-administered ethanol and, in broader terms, the proposed role of the NAcc as a limbic–motor interface (29,38,42,49,53).

When amphetamine is microinjected into the NAcc, changes in ethanol self-administration patterns are characterized by a decreased response rate that is evident from the onset of the operant session while the duration of total session responding is increased (25,52). The termination of ethanol-maintained responding induced by intra-NAcc raclopride infusions also occurs rapidly after the onset of responding. The early changes in operant responding observed after altering dopaminergic activity in the NAcc suggest that it is unlikely a direct pharmacological interaction between NAcc dopamine and the self-administered ethanol is responsible for the alterations in ethanol self-administration patterns. Given that ethanol reinforcement (25,52) and conditioned reinforcement (30,31,59,60,66) appear to be similarly modulated by NAcc dopaminergic activity and intragastrically self-administered ethanol can establish a CS^R (56), an alteration in the efficacy of CS^Rs associated with ethanol reinforcement could be partially responsible for the rapid onset of amphetamine's effects on patterns of ethanol self-administration.

An interaction of amphetamine and CS^Rs early during ethanol self-administration sessions is supported by the ability of NAcc dopamine and CS^Rs to influence appetitive behaviors (24,29,48,49,67). Manipulations that decrease dopaminergic activity in the NAcc impair appetitive behaviors while leaving the consequent consummatory behavior unaltered (29,48). For example, blockade of D_2-receptors in the NAcc has been reported to decrease running speed in a J-maze but consumption of a sucrose solution upon entry to the goal box is unchanged (29). With respect to CS^Rs, Hill (24) reported that responding during extinction could be reinstigated when a CS^R was presented contingent on lever pressing (24). Samson and Hodge (53) have proposed that ethanol self-administration is influenced by both appetitive and consummatory behaviors. Appetitive behaviors are hypothesized to influence the onset and early maintenance of a drinking episode. Therefore, an interaction between CS^Rs and dopamine in the NAcc could be hypothesized to influence responding during the early portions of an ethanol self-administration session due to the influence of appetitive behaviors on the onset and early maintenance of ethanol drinking.

The purpose of the present study was twofold. First, it was intended to extend the findings of Smith et al. (56), with regards to conditioned reinforcement, from intragastrically to orally self-administered ethanol. The demonstration of a CS^R associated with orally self-administered ethanol would have important implications for the development of treatment strategies for alcohol abuse (33,34,57). Second, the effect of increasing NAcc dopaminergic activity on responding maintained by the stimulus complex was examined. Evidence that increasing dopaminergic activity in the NAcc increases responding maintained by a CS^R associated with ethanol would further suggest that Cs^Rs play a role in the effects of intra-NAcc amphetamine on responding maintained by orally self-administered ethanol.

METHOD

Animals

Six male Long–Evans rats (Harlan–Sprague–Dawley; Indianapolis, IN) were used in this study. At the start of the experiment the rats ranged in weight from 206–214 g (mean = 210.33 g ± 3.61 SD). The rats were housed individually in standard hanging cages with food and water available ad lib except when noted below. The colony room in which the rats were housed was maintained on a 12-h light:12-h dark cycle (lights on 600 h). Animal care was in accordance with NIH guidelines.

Apparatus

Ethanol self-administration sessions were performed in modular operant chambers (Med-Associates; East Fairfield, VT). Each operant chamber (30 cm (l) × 30 cm (w) × 24.5 (h) cm) was equipped with two levers, two stimulus lights (75 watts), an automatic dipper, a houselight, a sonalert (Mallory Model #SC268F), and a stainless steel drinking tube. The automatic dipper was located in the center of the front wall of the chamber. The houselight was mounted 2 cm below the ceiling on the back wall of the chamber. A drinking tube, which was wired to the grid floor of the chamber, was located to the left of the dipper. Contact with the drinking tube closed an electrical circuit. When this circuit was closed, a licking response was registered by the computer. To the right of the dipper, a removable lever was mounted with a sonalert and stimulus light mounted above it. The sonalert (2900 ± 500 Hz, 75–85 dB) was connected in series to a 33 KΩ resistor operated by 24 volts DC. The second lever was mounted on the back wall of the chamber in the corner opposite the first lever. Each operant chamber was housed in a sound-attenuating enclosure. A fan mounted inside the enclosure masked external noise. All inputs and outputs of the chambers were controlled by an IBM compatible PC using Med-Associates software (Med-Associates; East Fairfield, VT).

Drugs and Solutions

d-Amphetamine sulfate (Sigma Chemicals, St. Louis, MO) was prepared immediately prior to each test session in artificial cerebrospinal fluid (aCSF). The aCSF vehicle had a pH of 7.4. The composition of the aCSF was: 10 mM dextrose, 1.14 mM potassium phosphate (KH_2PO_4), 1.21 mM magnesium sulfate ($MgSO_4$), 25 mM sodium bicarbonate ($NaHCO_3$), 2.6 mM calcium chloride dihydrate ($CaCl_2\ 2H_2O$), 4.7 mM potassium chloride (KCl), and 120 mM sodium chloride (NaCl). The 3% phosphate-buffered saline (PBS) used for perfusion had pH 7.4 and the composition: 122 mM sodium phosphate dibasic ($NaHPO_4$), 15 mM sodium phosphate monobasic (NaH_2PO_4), and 123 mM sodium chloride (NaCl). The sodium pyrophosphate buffer used for the blood ethanol determinations was composed of: 7.4 mM sodium pyrophosphate

($Na_4P_2O_7$), 7.6 mM semicarbazide, 22 mM glycine, and 10 mls of 2N sodium hydroxide/300 ml buffer.

Stereotaxic Surgery

Each rat was anesthetized with intraperitoneally (IP) administered sodium pentobarbital (40 mg/kg). Anesthesia was supplemented with IP chloral hydrate (137.5 mg/kg). When sedated, the rat was placed in a Kopf Stereotaxic device (Model #1404) with the nose bar adjusted to −3.3 mm. Stereotaxic coordinates for the nucleus accumbens were: A-P = +1.5 min, M-L = +1.8 mm, D-V = −6.0 mm (41). After making bore holes in the skull, 13-mm stainless steel 26 gauge cannulae guides were implanted into both sides of the brain. Four stainless steel screws and cranioplastic cement (Plastics One; Roanoke, VA) were used to secure the guides to the skull. After allowing the cement to dry, 33 gauge stainless steel solid obturators were placed into the guides. The obturators were replaced daily.

Site-Specific Nucleus Accumbens Microinjection

During each microinjection, the rats were held in small plastic containers (27 × 17 × 12 cm). The obturators were removed and stainless steel injectors (33 gauge) that extended 1 mm past the end of the guide cannulae were inserted by hand into the cannulae guides. The injectors were attached to 1 μl Hamilton syringes via PE-20 polyethylene tubing. The syringes were driven by Harvard Apparatus Infusion Pumps (Model 22). A volume of 0.5 μl/brain side was infused at a rate of 0.5 μl/min. After each injection, the injectors remained in place for 30 s before removal of the injector and replacement of the obturator. The rats were then placed in individual carriers for 10 min before the operant session was begun. Each dose effect curve consisted of a no injection control, sham injection, and three doses of amphetamine (5 μg/μl, 10 μg/μl, and 20 μg/μl). A single determination for the aCSF vehicle was used in each rat. To acclimate the rats to the microinjection procedure while maintaining the integrity of the brain tissue at the injection site, sham injections were performed prior to ethanol self-administration sessions before new response testing was begun. During sham injections, a 13-mm injector (equal in length to the cannula) was used so the brain tissue was not penetrated. The pumps were run but the syringes were not driven.

Blood Sample Collection and Blood Ethanol Determination

Immediately following the last ethanol self-administration session, each rat was restrained and a 100 μl blood sample from the tip of the tail was collected in a heparinized capillary tube. Evaluation blood ethanol levels was accomplished by enzymatic analysis (4).

Perfusion and Histology

Immediately following collection of blood samples, a lethal dose of sodium pentobarbital (100 mg/kg) was administered to each rat. When fully sedated, the rat was transcardially perfused with 60 ml of PBS. Perfusion with PBS was followed by perfusion with 60 ml of 10% formalin and removal of the brain. Each brain was stored in 10% formalin for 5–7 days until sectioned. The brains were cut into 90-μm sections with a sliding microtome and mounted on gelatin coated slides. The brain sections were allowed to fix in 10% formalin vapor for 7–10 days. The sections were stained with cresyl violet, dehydrated in an ascending series of alcohols (50% ethanol–100% ethanol) and cover slipped. Visualization of the injection site was determined with the aid of a light microscope.

Procedure

Upon arrival to the laboratory, the rats were weighed and handled for 1 week to allow for adaptation to the housing conditions. Adaptation was followed by 3 days during which time 10% ethanol (v/v) was the only fluid available for consumption in the home cage (i.e., Forced Ethanol Test). The forced ethanol test was followed by 14-day two-bottle home cage drinking test as described by Li et al. (35). Briefly, for 14 days two fluid sources (water and 10E) were available continuously in the home cage. Every day the volume of each fluid consumed during the previous 24-h period was recorded. The grams/kilogram (g/kg) of ethanol consumed and an ethanol preference ratio (ml 10% ethanol/ml total fluid) was then calculated. The fluid bottles were then refilled and placed back on the home cage. To prevent side preferences, the position of the water bottle on the home cage with respect to the ethanol bottle (left or right) was alternated daily.

Following completion of the home cage drinking tests, operant training began. The rats were fluid restricted for 12 h before the first of three overnight sessions. During overnight training a single lick response (fixed ratio 1, FR1) on an empty drinking tube resulted in 3-s presentation of 0.1 ml of 20% (w/v) sucrose from the automatic dipper. Responses emitted during the 3 s of ethanol presentation were recorded but had no programmed consequences. Levers were not present in the operant chamber during ethanol self-administration sessions. After the first overnight training session ad lib fluid access in the home cage was reinstated and maintained for the remainder of the experiment. When stable responding was established during overnight sessions (>500 lick responses/night), 30-min daily operant sessions began to be conducted 5 days/week (Monday–Friday). A modified sucrose-substitution procedure (51) was employed to initiate ethanol self-administration. Over a 14-session period the following solutions were employed as reinforcers: 10% sucrose (10S)—two sessions, 10% sucrose/2% ethanol (10S2E)—two sessions, 10% sucrose/5% ethanol (10S5E)—two sessions, 10% sucrose/10% ethanol (10S10E)—two sessions, 5% sucrose/10% ethanol (5S10E)—two sessions, and 2% sucrose/10% ethanol (2S10E)—two sessions. All sucrose solutions were prepared weight/volume and all ethanol solutions were prepared volume/volume. Next, 10% ethanol (10E) was employed as the reinforcer for five sessions. Over the next 10 sessions the schedule of reinforcement for the licking response was increased to a Fixed Ratio 10 (FR10). For the remainder of the experiment, an FR10 schedule was employed during ethanol self-administration sessions. Following five sessions of stable responding (<20% within-subject variation in responding) on an FR10 maintained by 10E, ethanol concentration manipulations were performed. During ethanol concentration manipulations, the concentration of ethanol presented as the reinforcer was increased to 15% (15E), 20% (20E), and 30% (30E) for five sessions each. Then, following the reestablishment of responding maintained by 10E, 26 gauge stainless steel guide cannulae aimed at the nucleus accumbens (NAcc) were implanted. Baseline responding maintained by 10E was reestablished prior to testing for the development of a new operant response (i.e., lever press) reinforced by the presentation of only a tone-light stimulus complex which had been previously paired with ethanol presentation.

Pairing of the tone–light stimulus complex with ethanol

presentation began during the sucrose-substitution procedure. During the first session when 10S2E was employed as the reinforcer, a tone–light stimulus complex (tone on–light on) was presented simultaneously and overlapping with each reinforcer presentation. Stimulus complex presentation was paired with all reinforcer presentations that contained sucrose/ethanol or ethanol for the duration of the experiment.

During each 1-h new response session, the drinking tubes were removed and two levers were mounted in each chamber. Completion of an FR1 on one lever (contingency-associated lever) resulted in the presentation of the tone–light stimulus complex, while no programmed consequences were associated with lever presses on the alternate lever (no contingency-associated lever). Ethanol reinforcement was never presented during new response test sessions. The lever designated as the contingency-associated lever was counterbalanced across the rats. The lever designation for each rat was only changed once during the course of the new response testing (see below). New response sessions were always separated by at least one 10% ethanol self-administration session (i.e., lick response reinforced according to an FR10) with no levers present in the chamber. The first new response session was employed as a no injection control session. In subsequent new response sessions, aCSF or amphetamine in aCSF vehicle was infused into the NAcc 10 min prior to each session. Amphetamine was administered in the following order: 20 μg/μl, 10 μg/μl, 5 μg/μl, 10 μg/μl, 5 μg/μl, 20 μg/μl. Following double determination of the amphetamine dose–effect curve, 10 μg/μl amphetamine was administered for a third time but the levers that had previously been designated as the contingency-associated lever and no contingency-associated lever were reversed (reversal condition). A final control new response session was performed after all amphetamine infusions were completed. Following completion of the new response portion of the experiment 2 additional weeks of ethanol self-administration sessions were performed. During this time, a blood sample was collected from each rat immediately after the completion of an ethanol self-administration session for analysis of blood ethanol levels. Following blood collection, each rat was sacrificed and the brain was collected.

Data Analysis

Data are reported as the average ± standard error of the mean unless otherwise noted (SD = standard deviation). During ethanol self-administration sessions, the number of responses emitted and reinforcers presented was recorded. From these data the number of stimulus complex presentations and total session ethanol intake in g/kg were determined. Individual within subject one-way repeated measures (RM) analyses of variance (ANOVAs) were used to analyze changes in responding, ethanol intake and stimulus complex presentations during ethanol self-administration sessions. Paired *t*-tests were used to determine if licking responses, ethanol intake or stimulus complex presentations during ethanol self-administration sessions changed after new response testing began. To analyze responding on each lever (contingency-associated lever vs. no contingency-associated lever) across the new response test conditions (control, sham injection, 0.0–20.0 μg/μl amphetamine) during testing, a Freidman's ANOVA on Ranks was employed, as the data were not found to be normally distributed. When necessary, a Student–Newman–Keuls multiple comparisons test was used for post hoc analysis. All statistics were performed with a commercially available statistical software package (SigmaStat for Windows; Jandel Scientific).

RESULTS

Histology

Histological analysis verified bilateral injection sites in the NAcc of all rats (Fig. 1). Injection sites ranged from +0.70 to +1.20 mm in the anterior–posterior plane from bregma. The injections sites were clustered around the anterior commissure and were classified as being primarily located in the core region of the NAcc.

Ethanol Self-Administration

At the end of the experiment, the average weight of the rats had increased to 594 ± 48 (SD) g (range = 522–647 g). Average ethanol intake during the forced ethanol test was 6.51 ± 3.34 g/kg/day. During the 14-day two-bottle test, average ethanol intake was 1.58 ± 0.25 g/kg/day. The average ethanol preference ratio was 0.16 ± 0.02. An average consumption of 5.3 ± 0.5 ml of ethanol and 28.9 ± 0.8 ml of water was recorded over the 14-day test period. These data are similar to those previously observed in this laboratory (51).

The number of licking responses significantly decreased, $F(11, 71) = 8.26$, $p < 0.001$, when 10E was employed as the reinforcer in comparison to 10S10E (Fig. 2, left top, open bars, FR1 schedule). No significant differences in reinforcer-stimulus complex pairings were observed between solutions that

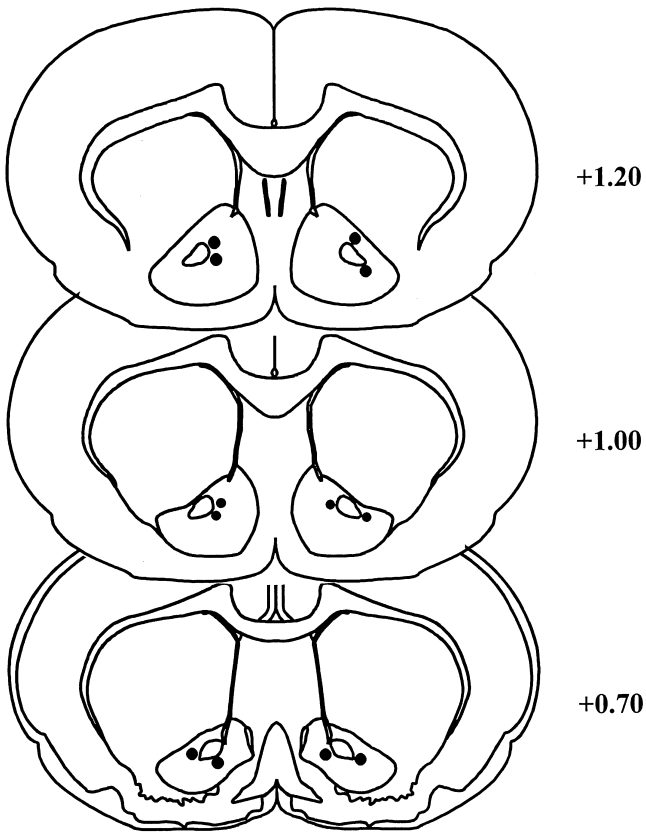

FIG. 1. Schematic coronal rat brain sections depicting injection sites as visualized by light microscopy. Each filled circle represents a single injection site. The values to the right of each section represent the approximate anterior distance in millimeters from bregma as determined from the atlas of Paxinos and Watson (41).

contained 10S; however significant concentration dependent decreases, $F(11, 71) = 23.6$, $p < 0.001$, in the number of pairings were observed as the sucrose concentration decreased from 10–0% (Fig. 2, left bottom, filled bars, FR1 schedule). Ethanol intake followed an inverted U-shaped function as the sucrose–ethanol solutions were manipulated during the sucrose substitution procedure. Ethanol intake when 10S10E was the reinforcer was significantly greater, $F(11, 71) = 16.3$, $p < 0.001$, than all other solutions employed except 5S10E (Fig. 2, left top, filled circles, FR1 schedule). The data indicate that responding continued to occur during reinforcer presentation because the number of reinforcer–stimulus complex presentations was less than the number of responses emitted while the FR1 schedule was in effect (Fig. 1: top, open bars, left vs. bottom, filled bars, left).

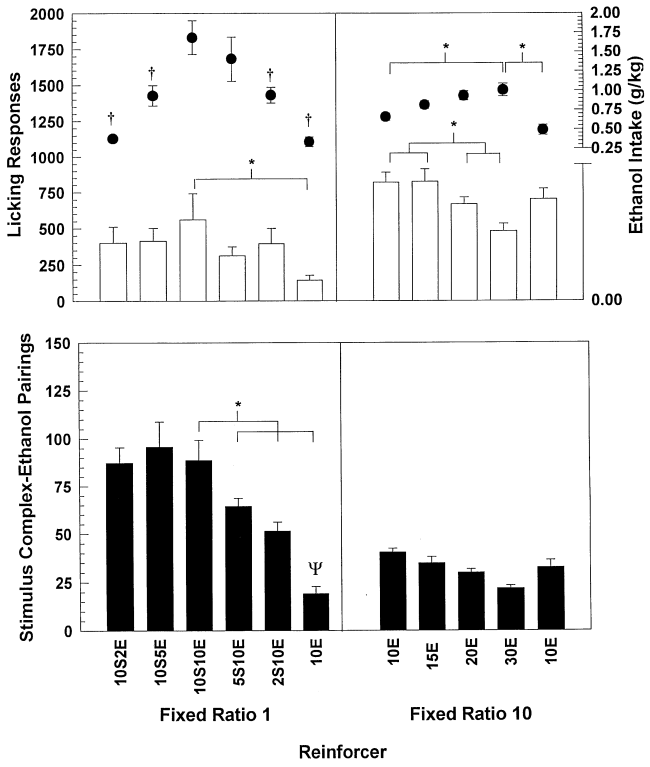

FIG. 2. Average licking responses and ethanol intake (top) and ethanol-stimulus complex pairings (bottom) during the sucrose substitution (left) and ethanol concentration manipulation (right) phases of the experiment. During the sucrose substitution procedure (left) all responding was maintained on a fixed ratio 1 schedule of reinforcement. During the ethanol concentration manipulations (right) all responding was maintained on a fixed ratio 10 schedule of reinforcement. Each point is the average of all rats ($n = 6$). Error bars are SEM. Filled circles represent ethanol intake in grams/kilogram (g/kg). Open bars represent average total session responses and filled bars represent the average number of ethanol-stimulus complex pairings when each reinforcer was presented. *Represents significant difference ($p < 0.05$) between bracketed bars. †Represents significant difference from 10% sucrose/10% ethanol. ψRepresents significant difference from all other reinforcers (10% sucrose/2% ethanol–2% sucrose/10% ethanol). x-axis labels refer to reinforcer presented contingent on completion of the operant task: 10S2E–10% sucrose/2% ethanol, 10S5E–10% sucrose/5% ethanol, 10S10E–10% sucrose/10% ethanol, 5S10E–5% sucrose/10% ethanol, 2S10E–2% sucrose/10% ethanol, 10E–10% ethanol, 15E–15% ethanol, 20E–20% ethanol, 30E–30% ethanol.

The number of licking responses when 20E and 30E were reinforcers was significantly less, $F(11, 71) = 8.26$, $p < 0.001$, than the number or responses with 10E and 15E reinforcement (Fig. 2, right top, open bars, FR10 schedule). Responding increased back to initial 10E levels when the reinforcer was returned to 10E following 30E. Decreases in responding as the ethanol concentration increased were paralleled by nonsignificant decreases in the number of stimulus complex–ethanol parings (Fig. 2, right bottom, open bars. FR10 schedule). The number of pairings returned to baseline levels when 10E was restored as the reinforcer. As the ethanol concentration increased from 10–30%, a significant increase, $F(11, 71) = 16.3$, $p < 0.001$, in ethanol intake was observed (Fig. 2, right top, filled circles) with intake at 30E being greater than 10E. A significant decrease in ethanol intake was then observed when the ethanol concentration returned to 10E. Ethanol intake was not statistically different between the two 10E determinations. As was observed during the FR1 schedule, responses were emitted during reinforcer presentation while the FR10 schedule was in effect (Fig. 1: top, open bars, right vs. bottom, filled bars, right). The changes in total session responding during the ethanol concentration manipulation phase of the experiment, as well as the average ethanol intake at each concentration, are similar to those previously reported when a lever press response was employed (51).

New Response and Amphetamine Microinjection

Prior to new response testing the average total number of stimulus complex–ethanol reinforcer pairings for each rat was 2912 ± 631 (SD) over the course of 74 operant sessions (range = 2110–3930). A paired *t*-test revealed that performing new response sessions did not result in significant changes in licking responses during the interspersed ethanol reinforced sessions ($t = -1.43$, $p = 0.21$). After beginning new response testing, licking responses emitted during the interspersed 10E self-administration sessions averaged 851 ± 43. 10E-maintained lick responses immediately prior to the start of new response testing averaged 700 ± 73. Measures of ethanol intake (during new response testing period = 0.54 ± 0.07, prior to new response testing period = 0.49 ± 0.07; $t = -0.474$, $p = 0.65$) and the number of stimulus complex pairings per session (during new response testing period = 38 ± 4, prior to new response testing period = 33 ± 4; $t = -0.483$, $p = 0.65$) were also not statistically different prior to and after the start of new response testing.

No significant differences in lever press responses were observed between repeated determinations of each experimental condition during new response test sessions (Fig. 3). The data presented are the average of two determinations for each condition with the exception of the vehicle injection and reversal conditions for all rats ($n = 6$). Significant changes in the median level of lever pressing were found between experimental conditions ($\chi^2 = 35.8$, $p < 0.001$). No significant differences in the number of lever presses between levers were observed within or between nonamphetamine testing conditions (control, vehicle injection, sham injection). Statistically significant increases in the number of lever presses were observed on the stimulus complex-associated (contingency-associated) lever after administration of all doses of amphetamine with the exception of when the lever designations were switched (reversal + 10 μg/μl amphetamine). Only nonsignificant increased trends were observed on the no contingency-associ-

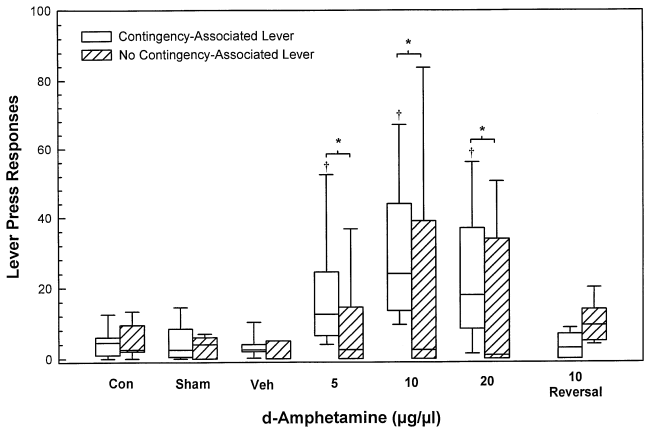

Fig. 3. Box plot depicting lever press responses during each 1 h new response session. Each open bar represents responding on the lever that resulted in presentation of the tone–light stimulus complex (contingency-associated lever). Each diagonally crosshatched bar represents responding on the alternate lever (no contingency-associated lever). Error bars represent 10th to 90th percentiles. The box encompasses the 25th to 75th percentiles. The horizontal bar through each bar represents the median. X-axis represents responding under control (Con), sham, vehicle (Veh), amphetamine microinjection (5, 10, 20 µg/µl), or the reversal condition (10 reversal). Each bar represents all subjects ($n = 6$). * Represents significant difference between brackets ($p < 0.05$). † Represents significant difference from control, sham, and vehicle conditions on the same lever ($p < 0.05$).

ated lever following amphetamine infusion (Fig. 3). The range of responding (10th–90th percentiles) on both the contingency-associated and no contingency-associated lever increased following intra-NAcc amphetamine infusion.

Blood Ethanol Analysis

Due to differences in the patterns of ethanol self-administration, the time between the final reinforcer presentation and blood sampling ranged from 5–25 min despite immediate blood collection following completion of the self-administration session. Blood ethanol levels ranged from 0.00 to 30.85 mg% (mean = 8.45 ± 4.99). Ethanol intake on the day that blood samples were collected ranged from 0.30–0.83 g/kg (mean = 0.60 ± 0.08).

DISCUSSION

The present study examined whether a stimulus complex paired with oral ethanol self-administration could be established as a CS^R. Using a sucrose-substitution procedure and ethanol concentration manipulations, the self-administration of ethanol was initiated while a stimulus complex was associated with ethanol presentation. Similar ethanol intakes were observed during this study in comparison to those previously observed in this laboratory (25,51,52). The lack of food- or fluid-restriction in these rats suggests that the maintenance of ethanol self-administration by caloric- or fluid-related factors were minimal. In addition, although the measured blood ethanol levels were low, these data suggest the ethanol solutions were being consumed. Pairing of the stimulus complex with ethanol presentation did not result in the ability of the stimulus complex to maintain lever pressing in the new response paradigm (control, vehicle injection, sham injection) except after activation of the mesolimbic dopamine system by intra-NAcc amphetamine infusion. This suggests that the stimulus complex was capable of functioning as a CS^R only when dopaminergic activity in the NAcc was augmented.

In agreement with previous reports (30,31,59,60,66), intra-NAcc amphetamine administration produced significant increases in responding on a lever associated with presentation of the paired stimulus complex. The lack of a statistically significant increase in responding on the no contingency-associated lever in the present study suggests that the increases in responding were not due to nonspecific increases in locomotor activity. The absence of a significant increase in responding on either lever following amphetamine infusion when the lever designations were switched further indicates that the amphetamine induced increases in responding are context dependent. It also indicates that there was some location-specific enhancement of the effectiveness of the CS^R during prior amphetamine testing. Changing the location of the contingency-associated lever was sufficient to block the increases in responding observed after amphetamine administration. Although the reversal test was only a single determination and the 8th drug infusion, it is unlikely that the lack of an amphetamine effect was due to damage at the injection site. If this had occurred, differences in the repeated dose determinations between the two 20 µg/µl infusions (1st and 7th infusions) would have been expected. No decrease was observed between these injections, and it is unlikely that all sensitivity to amphetamine would have been lost between the 7th and 8th infusions. Numerous studies have reported that the increases in responding induced by amphetamine are specific to stimuli paired with reinforcer presentation and are not observed when randomly paired or unpaired stimuli are presented contingent on responding (2,3,27,37,45,46,47,59). The exceedingly low amount of lever pressing maintained by the stimulus complex in this study under no-drug conditions, in conjunction with the low amount of lever pressing maintained by similar stimuli prior to pairing with reinforcement (3,45,46), suggests that these type of stimuli have minimal, if any, inherent reinforcing properties. Furthermore, these effects are attributed primarily to altered dopaminergic activity within the NAcc because prior studies have demonstrated that amphetamine's effects on CS^Rs when infused into the NAcc are neurochemically specific to the dopaminergic system (9,60). Thus, the data suggest that the stimulus complex functioned as a CS^R after microinfused amphetamine increased dopaminergic activity in the NAcc.

Although the nature of NAcc dopamine, ethanol self-administration, and CS^R interactions are not well understood, it is hypothesized that a CS^R would influence the onset and early maintenance of an ethanol drinking episode prior to the onset of ethanol's pharmacological actions. However, alterations in ethanol-reinforced lever pressing after intra-NAcc amphetamine infusions are maintained for the entire session (25,52). This suggests that either additional stimuli are being impacted by enhanced NAcc dopamine or the behavioral control of an ethanol drinking episode by CS^Rs is not restricted to the early portions of the drinking episode. The minimal amount of behavior maintained by the stimulus complex in this study during the new response sessions under no-drug conditions made it impossible to discern changes in the pattern of responding after intra-NAcc amphetamine infusions. Therefore, the interaction between conditioned reinforcers and ethanol self-administration needs to be examined further in a paradigm that generates more behavior.

Although these data are in agreement with previous reports on the effects of intra-NAcc amphetamine infusion on

responding maintained by CS^Rs (30,31,59,60,66), the data obtained during no-drug conditions (control, vehicle injection, sham injection) suggest the stimulus complex did not function as an efficacious reinforcer by itself. Using the new response paradigm, responding maintained on one lever by a stimulus complex previously paired with a primary reinforcer has been reported to be greater than responding on an alternate lever with no programmed consequences (3,30,31,45,46,59,60,66). The inability of the stimulus complex to maintain responding during no-drug conditions in the present study might be attributed to a combination of procedural factors. Food restriction has repeatedly been shown to increase responding maintained by both caloric and noncaloric reinforcers (11,14,54). Given that the increases in responding of food restricted rats are generalizable to many types of reinforcers, it is plausible that responding maintained by a CS^R might also be augmented by food restriction. This hypothesis is supported by data obtained from Robbins and Koob (47) and Phillips et al. (43). The pattern of stimulus complex–ethanol pairings employed in this study was also unique in comparison to previous studies (3,30,31,45,46,59,60,66). A brief conditioning phase with an intermittent and delayed stimulus complex pairing is typically used to establish a CS^R (3,30,31,45,46,59,60,66). The pairing procedure in the present experiment was considerably longer in duration (74 sessions) and the stimulus complex was paired simultaneously with all ethanol presentations resulting in more than 2000 stimulus complex–ethanol pairings. The high frequency and number of pairings may have attenuated the salience of the stimulus complex in this study, reducing the potential for it to function as a CS^R. This possibility is supported by studies that have employed behavioral paradigms with prolonged training periods (25–60 sessions) to study conditioned reinforcement (16).

Alternatively, the type of stimulus complex (visual–auditory) employed may not be the most effective in eliciting conditioned responding associated with alcohol drinking. Studies of the reactivity of alcoholics to simple visual stimuli and visual–olfactory stimulus complexes associated with ethanol have reported that these stimuli are sufficient to act as conditioned stimuli (22,34,40,57,61), but the taste of ethanol or the ingestion of small priming doses of alcohol are considerably more effective (19,33,61). The present study attempted to simulate the pattern of pairing that might occur during a natural drinking episode in humans by employing consistent and long-term pairing of a compound visual–auditory cue with ethanol consumption. These data would suggest that in alcohol users not all cues associated with alcohol consumption elicit conditioned responses to the same degree. Although visual, olfactory, and taste cues may all be paired with ethanol consumption, certain types of cues appear to be more salient in the drinking situation, and thus, may be more effective at becoming CS^Rs.

In his classic studies, Garcia et al. (18) reported that flavor cues were highly effective in establishing conditioned taste aversions (CTA) to foods when those flavors were paired with x-ray–induced illness, but pairing a specific food pellet size with x-ray–induced illness was not sufficient to establish a conditioned aversion to that size food pellet. In contrast, association of a flavor with foot shock was not sufficient to produce a CTA but consumption of a specific food pellet size could be decreased when that pellet size was paired with foot shock. In light of these data, a potential explanation for the stimulus complex not functioning as an effective CS^R could be that other stimuli were more salient during the conditioning procedure. Taste (12,17,18,21,28,36) and odor (39) stimuli have been reported to serve as highly effective conditioned stimuli in classical conditioning paradigms. Taste may have been a particularly salient cue in this study due to the use of sucrose substitution initiation procedure. This method of ethanol initiation acclimates the rat to a progressively more intense ethanol taste as the concentration of ethanol increases and/or sucrose decreases during training. Thus, an ethanol taste cue may be a salient stimulus that impacts patterns of ethanol self-administration. A conditioned taste cue could also be hypothesized to alter behavior at the start of the session when the reinforcer is first sampled. Therefore, an examination of the ability of taste to influence ethanol self-administration warrants further study.

The NAcc has been proposed to function as a limbic–motor interface (29,38,42,49,53). The extensive afferent and efferent connectivity of the NAcc (23,42,68) make it well suited for integrating input from a variety of limbic structures including the hypothalamus, hippocampus, prefrontal cortex, and amygdala. The execution of appropriate motor behaviors in response to salient stimuli in the environment (internal or external) can thus be influenced via its efferent connections to the motor systems. The mesolimbic dopamine system has been implicated in a variety of processes potentially related to stimulus processing. Therefore, the effects of NAcc dopaminergic activity on conditioned reinforcement are most likely not restricted to reinforcement processes. Changes observed in locomotor activity (6,10,15), prepulse inhibition (63), and switching behavior (62) after intra-NAcc amphetamine infusion or passive avoidance learning after 6-hydroxydopamine lesions of the NAcc (55,58) all suggest a broader function for the mesolimbic dopamine system. Although a variety of limbic structures may be important in assigning "incentive-motivational" valence to a particular stimulus (5,7,8,64), the role of the NAcc in the mesolimbic circuit appears to be the processing of information relating to the presence of salient stimuli and eliciting the appropriate action to those stimuli. The modulation of conditioned reinforcement by dopamine in the NAcc supports the hypothesis.

In summary, these data suggest that a stimulus complex paired with oral ethanol self-administration can function as a CS^R when dopaminergic activity in the NAcc is increased. The ability of the stimulus complex to function as a CS^R suggests that other stimuli (i.e., taste) closely associated with ethanol consumption may function in a similar fashion after prolonged ethanol consumption. Taste cues have been reported to alter ethanol craving in humans (19,33,61). The regulation of patterns of ethanol self-administration by taste cues, therefore, needs to be more fully elucidated in animal models of ethanol self-administration. Given the increased responding maintained by the stimulus complex after intra-NAcc amphetamine infusion, the effects of intra-NAcc amphetamine on oral ethanol self-administration could be partially attributed to increased control of responding by CS^Rs associated with ethanol.

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